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Effects of Allylestrenol on Blood Lipids in Relation to its Biological Activity

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Allylestrenol (AE), a progesterone derivative, is used in replacement therapy as a contraceptive steroidal hormone. Considering the importance of its partition coefficient parameter (log P= 6.13), a significant contributor to its action mechanism, interaction of the drug with blood lipids and related fatty acid changes along with peroxidation phenomena have been investigated to explain its reported mode of action. From the present investigation, it was observed that lipid loss after incubation of whole blood with AE for varying periods of time was accompanied with significant changes in fatty acid composition. It was also found that AE caused a significant extent of lipid peroxidation. Ascorbic acid (AA), an antioxidant at equivalent human dose of 250 mg could significantly reduce AE-induced lipid peroxidation.

Interaction of a drug with blood lipids correlates well with the partition coefficient parameter[1] of the drug and the biological response[2], either therapeutic or toxic. These responses involve a chain of physicochemical events within the biophase at the receptor site[3,4] where the drug binds through hydrophobic and/or other types of interactions. The changes occurring in the cell membrane during partitioning of a drug in various compartments include changes in membrane constituents, such as, i) binding with phospholipids of biomembranes, ii) changes in the fatty acid (FA) composition and iii) lipid peroxidation[5].

FAs are important constituents of biomembranes that control membrane fluidity[6] and are precursors of prostaglandins and other eicosanoids that regulate important body functions[7]. Hence, change in the lipid pattern seem to be highly important in drug action mechanism[8], Binding with phospholipids have been linked with therapeutic effects[9,10] and lipid peroxidation with toxic effects of the drug[5,11,12].

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MATERIALS AND METHODS

Blood collected from the goat *(Capra capra)*, chemicals of analytical grade and authentic samples of fatty acid methyl ester (FAME) obtained from V.P. Chest Institute, Center for Biochemical Technology, New Delhi were used in the present investigation. Fatty acid estimation was carried out at Regional Sophisticated Instrument Center, Bose Institute, Calcutta. Allylestrenol was provided by Infar (India) Ltd., Calcutta.

The experimental steps for determination of lipid loss and fatty acid composition are as follows : i) extraction of total lipid from blood was done with methanol-chloroform mixture by the method of Bligh and Dyer[13], ii) estimation of phosphorus in total lipid was done according to the procedure of Allen[14] and the color developed was measured using a colorimeter (EC model GS 5700A) at 680 nm, iii) saponification of whole blood lipid was done

with methanolic sodium hydroxide and the free fatty acids were converted to their corresponding methyl esters using methanolic hydrochloric acid (2.5%) according to the method of Kates[15,16], iv) the fatty acid methyl esters (FAMEs) were first purified by TLC[17] on silica Gel G-coated plates using n-hexane:diethyl ether:glacial acetic acid (80:20:0.25 %v/v) as solvent system. Then quantification of the purified FAMEs were done by GC (Pye Unicam model 104) equipped with FID and glass column packed with DEGS supported on 100-120 mesh chromosorb WAW and connected with SP 4270 integrator. The nitrogen flow rate was 60 ml/min in an oven maintained at temperatures 190°. Detector and injection port temperatures were 269° and 240° respectively. FAMEs were identified by using standard methyl palmitate and methyl stearate as primary standards and comparing the relative retention time of the sample peaks with those of standard FAMEs separated on the same column under identical operational condition.

Quantitative determination of lipid peroxidation end product, i.e., TBA-titres was done by the method of Tarladgis *et al.*[18,19] with some modification[20] using a colorimeter (E C model GS 5700A) at 530 nm. Lipid peroxidation induced by the AE and the protective role of AA in reducing the drug-induced lipid peroxidation were determined and compared with control samples.

In all these experiments, AE was used at 1.664×10^{-2} µmol/ml of blood concentration which is about 95-98% (maximum concentration in blood-serum) at a regular dose of 25 mg. Both the control and drug-treated blood samples were incubated for 24 h as the biological half-life of AE is about (10±4) h and determination of various parameters mentioned above was performed at 2nd, 6th, 10th and 24th h. To suppress AE-induced lipid peroxidation, AA was used at two equivalent human dose levels of 250 mg and 500 mg, i.e, the effective concentrations[21] are 28.4 µmol/ml 56.8 µmol/ml and of blood respectively.

RESULTS

The estimated inorganic phosphorus contents of different samples were compared to that of control sample (0 h incubation). The control sample did not show any significant change in phosphorus content in different time periods. Fig. 1 shows the relative percentage reduction in inorganic phosphorus content in AE-treated blood samples. The results show that the average loss of phosphorus in whole lipid due to drug effect is significant from

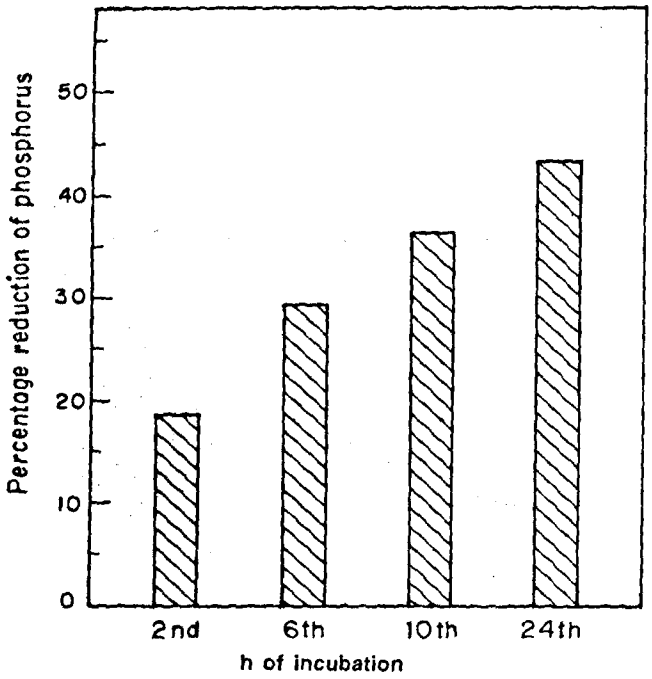

Fig. 1 : Average[1] percentage reduction of phosphorus in blood lipids due to allylestrenol.

[1]Mean of 5 animal sets. Statistical comparison was made between test and control values by 't' test., The values are significant at $P \leq 0.001$

2nd to 24th h of incubation. This may be accounted due to phospholipid binding capacity of the drug.

The relative percentage changes with respect to control in FAMEs of total lipids due to drug effect are presented in Fig. 2. There is no significant change of saturated fatty acid (SFA) content. Monounsaturated fatty acid (MUFA) content increases after drug administration while polyunsaturated fatty acid (PUFA) content decreases significantly from 2nd to 24th h of incubation. Fig. 3 represents the relative percentage changes of TBA (thiobarbituric acid)-titre (i.e., lipid peroxidation end products) due to drug alone and in combination with two concentrations of AA. The results show that the TBA-titres in AE-treated blood increases significantly in comparison to control in a time-dependent manner. AA at equivalent human dose 250 mg could significantly suppress the drug induced lipid peroxidation. When AA was used at equivalent human dose level of 500 mg, suppression of TBA-titre was not found till 24th h of incubation.

DISCUSSION

It is obvious from the results obtained from the

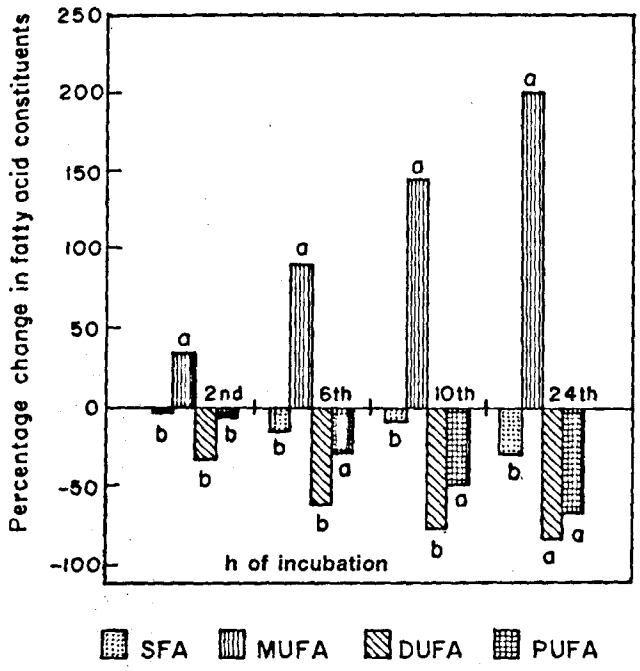

Fig. 2 : Average[1]changes in fatty acid constituents of blood lipids with AE.

[1]Mean of 3 animal sets. Keys: SFA, saturated fatty acid ($C_{14:0}$; $C_{16:0}$; $C_{18:0}$; $C_{20:0}$; $C_{22:0}$) ; MUFA, monounsaturated fatty acid ($C_{16:1}$; $C_{18:1}$; $C_{20:1}$) ; DUFA, diunsaturated fatty acid ($C_{16:2}$) ; PUFA, polyunsaturated fatty acid ($C_{18:3}$; $C_{18:4}$; $C_{20:4}$; $C_{20:5}$; $C_{22:3}$). Probability level (P) of changes are significant at a<0.05 and b>0.05.

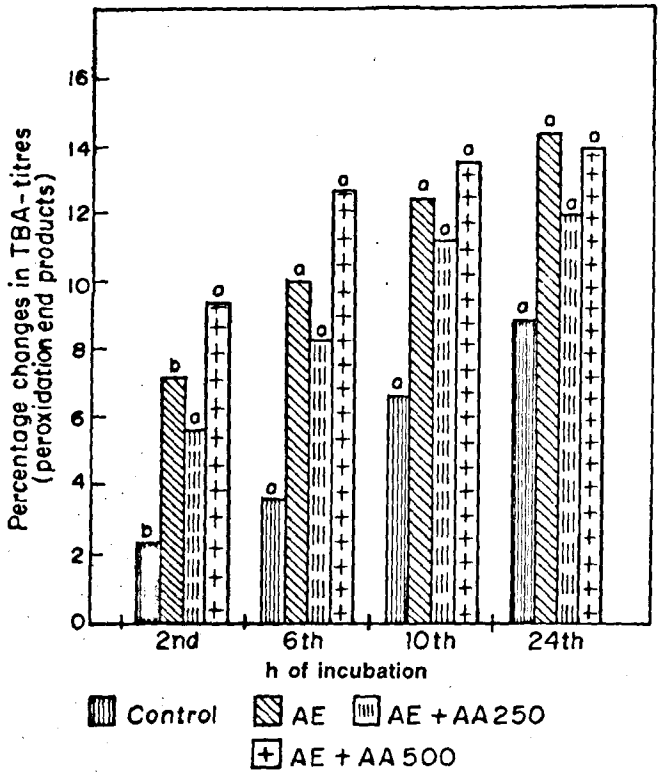

Fig. 3 : Average[1] percentage changes in TBA-titres induced by AE, AA 250[2] and AA 500[3].

[1]Mean of 5 animal sets, [2]Ascorbic acid at dose level 250 mg and [3]Ascorbic acid at dose level 500 mg., Statistical comparison were made between test and control values by 't' test., Probability level (P) of percent changes are significant at a<0.001 and b>0.001., The variance ratios (between samples and between animal sets) obtained from ANOVA are shown above the corresponding bar diagrams., Significance level of F values : between samples (d.f. 3, 12), P<0.001 and between animal sets (d.f. 4, 12), P>0.1.

present investigation that AE appreciably lowers the phosphorus content of the blood lipid due to binding with phospholipids because of its high lipophilicity[1] (log P=6.13) and this observation is corroborated by the fact that AE diffuses passively through cellular lipoidal membrane and binds to a progesterone receptor present in the nucleus[22]. The progesterone receptor is a ligand-activated nuclear transcription factor that interacts with a progesterone response element in target genes to regulate their expression[23]. AE may bind to the receptor with hydrophobic bonding, enhancing differentiation and opposing the actions of estrogens to stimulate cell proliferation. AE decreases PUFA content and *vis-a-vis* increases the MUFA content which is in good relation with binding of drug with phospholipid. The higher MUFA and lower PUFA content due to drug effect may be correlated with increase in lipid peroxidation end products because PUFAs are the precursors of eicosanoids, important physiological regulators[7] and may be converted to peroxide end products[24].

In lipid peroxidation, a hydrogen atom is liberated from the fatty acid by a reactive free radical resulting the formation of lipid radical[25] which on attack by molecular oxygen produces a lipid percxy radical forming either a lipid hydroperoxide or endoperoxide. The formation of lipid endoperoxide in unsaturated fatty acids (specially PUFA) leads to the formation of malonaldehyde (which is measured in terms of TBA-titres) as a break down product[26]. This increase in MUFA and decrease in PUFA level may be responsible for toxic effects of AE. As lipid peroxidation is a molecular mechanism of cell injury with potential injurious consequences[24], increase in TBA-titre due to AE may be related with its toxic potential. The

replacement therapy of progestin with AE increases the risk of thrombosis and other side effects including edema, abdominal bloating and headache[27] which may be consequences of generation of toxic peroxidation end products.

The fact that AA at a human dose level of 250 mg reduces formation of drug-induced peroxidation suggests that AA at lower dose level may have potential of reducing AE-induced toxicity on coadministration with drug. The increase in lipid peroxidation in AA-treated samples at higher dose may be due to the prooxidant effects of AA. AA reduces Fe^{3+} to Fe^{2+} through Fenton's reaction which promotes generation of free radicals and other highly reactive species accelerating lipid peroxidation[28,29]. Thus cotherapy with AA may be proved helpful in reducing iatrogenicity of drug if serum level of iron is carefully monitored.

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